The Endophytic Mycoflora of Bladder Saltbush (*Atriplex vesicaria* Hew. ex Benth.) and its Possible Role in the Plant's Periodic Decline.

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Periodic widespread death of bladder saltbush has been described in eastern Australia but not observed in similar communities west of the Eyre Peninsula. The dominant fungal flora of internal root and shoot tissues of bladder saltbush was assessed in 270 plants sampled from 35 sites in six chenopod communities across southern Australia. Seventy one species from 40 genera were isolated, of which *Fusarium equiseti*, *F. lateritium*, *F. nygamai* and *F. oxysporum* were the most frequent. *F. nygamai* and *F. oxysporum* were more common in roots from the eastern communities but there was no particular trend across regions in the relative frequency of fungal species from stems. There was no apparent relationship between subjective assessment of plant vigour and presence of these fungi. These fusaria could be re-isolated from plants inoculated in pathogenicity tests, although 'dieback' symptoms were not produced.

The majority of fungi isolated have not previously been recorded in association with *A. vesicaria* and this is the first published record of *Alternaria chlamydospora*, *Libertella* spp., *Phoma variospora* and *Sporormiella intermedia* in Australia. Several other known plant pathogenic genera (*Ascochyta*, *Coniothyrium*, *Phomopsis*, *Pleospora*) were represented in the endophytic mycoflora but there was no direct evidence that 'dieback' could be attributed to any single species. It is argued, however, that dieback may be a consequence of more extensive tissue invasion within plants by the internal microflora when plant vigour and host defence mechanisms are impaired by stress.

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INTRODUCTION

In the late 1970's and early 1980's stands of perennial bladder saltbush (*Atriplex vesicaria* Hew. ex Benth.) on the Riverine Plain in south-western New South Wales were widely affected by a disorder, of unknown aetiology, called saltbush dieback (Clift *et al.*, 1987). Symptoms varied from a progressive dieback of one or more branches of a bush to sudden death of individual bushes or clumps of bushes. Occasionally one stem of an otherwise healthy bush wilted with slight loss of colour in the leaves. Usually affected bushes shed leaves, but if they were retained, they turned sandy/olive in colour. During 1977-83, graziers frequently described this phenomenon beginning as scattered patches of dieback which expanded on a slowly advancing front (Clift *et al.*, 1987). Sometimes, however, it advanced rapidly across entire properties in a matter of weeks. During this period the area of bladder saltbush in western NSW declined by 53% (Clift *et al.*, 1987; Semple, 1989).

Dieback was not restricted entirely to bladder saltbush stands. Bluebush (*Maireana pyramidata* (Benth.) P. G. Wilson), cotton bush (*Maireana* spp.) and Old Man Saltbush (*Atriplex nummularia* Lindl.) stands were also affected to some degree. Bladder saltbush decline was most noticeable compared with that of other species because of the plants importance to graziers over extensive areas and the severity of dieback within this plant community.

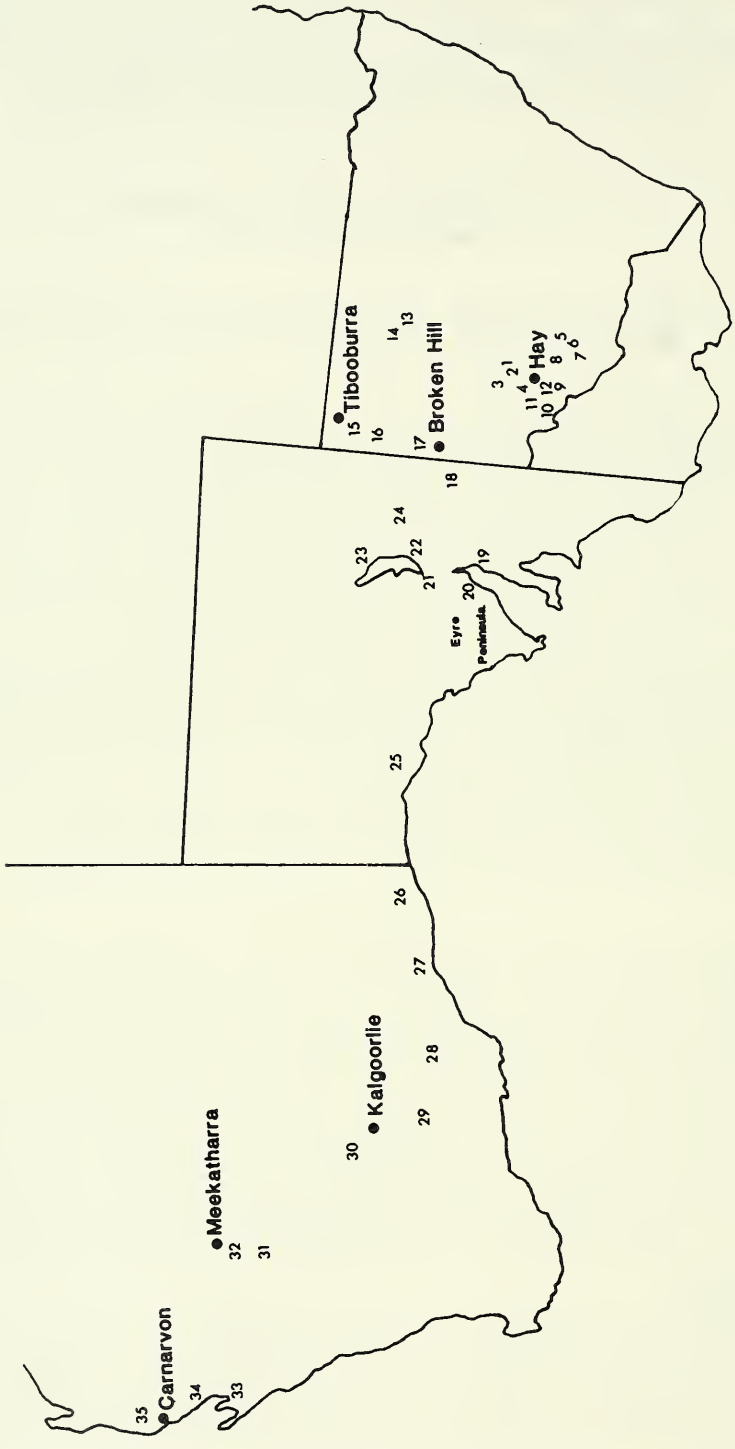

Fig. 1. Location of sampling sites within *Atriplex vesicaria* communities in southern Australia.

Perennial bladder saltbush is an important component of rangelands in the Western Division of NSW. The main value of saltbush is as a reserve fodder during dry periods when it contributes the bulk of stock diet in the hot dry summer (Leigh and Wilson, 1970). The loss of shrub through heavy grazing or other cause can substantially reduce carrying capacity (Wilson *et al.*, 1982). The resultant change in species dominance following drought increases the vulnerability of all remaining species to grazing (Charley, 1959) and the soil to erosion. *A. vesicaria* is one of the most important species in maintaining the stability of pastoral and natural ecosystems (Wilson and Graetz, 1979).

There are few records of widespread death of saltbush, but several early reports of 'dieback' associate it with insect activity (Semple, 1989). Graetz and Wilson (1984) noted, without comment, dieback in the absence of insects, while other observers have attributed the degraded nature of bladder saltbush communities to overgrazing and/or drought (Beadle, 1948; Leigh and Mulham, 1971). Other reports merely note a decline in saltbush density (e.g. Stanley, 1983). An investigation by Napier (1983) of dieback, in the absence of insects, of *A. undulata* and *A. rhagodioides* on degraded soils in W.A. found no conclusive evidence that plant pathogens were responsible. Although this phenomenon is mostly documented for the Riverine Plain of NSW, all saltbush stands as far west as the eastern edge of the Eyre Peninsula, South Australia, were affected to some extent during 1977-1983.

During 1985 to 1987, there were no new reports of widespread dieback. Stands of bladder saltbush were mostly classified as either dead or regenerating (Clift *et al.*, 1989). Bushes displaying 'classic' dieback symptoms occurred singly or as localized patches in otherwise healthy saltbush communities. Symptoms were the same as reported by landholders during the widespread dieback years which were described as leaf wilt rapidly followed by abscission. Although wilting can be readily evident on saltbushes, it is the rapid abscission of the leaf which distinguishes 'classic' dieback from death due to other causes (e.g. drought).

One noticeable symptom of dieback was sudden death of branches on otherwise healthy plants or sudden death of the entire plant. Specimens examined at the Biological and Chemical Research Institute, Rydalmere from 1980 to 1983 yielded several known plant pathogens (*Ascochyta caulina*, *Fusarium acuminatum*, *F. lateritium*, *Hendersonia sp.* (NSW Agriculture, Rydalmere Herbarium (DAR); unpublished) from necrotic tissue at the crowns of plants and from discoloured root tissue. This association, together with observations by the authors in 1983 of wilt symptoms in ungrazed bushes near Hay, suggested that plant pathogenic fungi may be associated with dieback. A detailed study was thus undertaken to isolate the fungi which may be involved in this phenomenon. This paper describes the dominant fungal flora associated with internal tissues of bladder saltbush in eastern Australia where dieback occurred, and in western regions where it has not been recorded. Other possible causes of saltbush dieback have been discussed by Cother *et al.* (1988) and Clift (1989).

Methods

Sampling

Bushes, identified as in decline, were sampled at random from 24 locations in NSW and eastern South Australia in 1985 and 1986 (Table 1, Fig. 1). Where possible apparently healthy bushes were also collected. Plants were also sampled at 'Tin Tin' Station, west of Oxley NSW, (site 10) in September 1985 and again in March 1986 to provide a comparison between bushes in spring and autumn. Bushes were classified prior to sampling, on the basis of foliage condition, as (a) healthy: green foliage with no

dead stems; (b) regenerating: some dead stems, regrowth of secondary cambium evident and greater than one third of bush possessing green leaves; (c) 'dead': few, usually bleached, leaves present, stems mostly dead but some green tissue evident when lower stems were cut. Entire bushes were sampled together with the root system occupying ca. 0.015m^3 of soil beneath the plant.

TABLE 1

Location of bladder saltbush communities sampled between July 1985 and October 1986

Geographic regions are based on the approximate boundaries of chenopod communities in Australia defined by Graetz and Howes (1979).

Site	Location	Geographic region	Sampling date
1	Mutherumbung Station, NNW of Booligal		7/85
2	Yamba Station, NW of Booligal		7/85
3	Freshwater Station, NW of Booligal		7/85
4	One Tree		7/85
5	Blacks Swamp		7/85
6	Caroonboon		7/85
7	Windouran Station, S of Maude		9/85
8	Everslie Station, S of Maude		9/85
9	Tchelery Soil Conservation Exclosure		9/85
10	Tin Tin Station, West of Oxley		9/85, 3/86
11	Kitcho Station, E of Hatfield		8/85, 5/86
12	Tupra Station, Oxley		9/85
13	75 km N of Wilcannia		9/85
14	37 km and 57 km W White Cliffs on Kayrunnera Road		9/85
15	Tibooburra	2	9/85
16	147 km S of Tibooburra on Broken Hill Road		9/85
17	30 km N of Broken Hill on Tibooburra Road		9/85
18	Bindy, adjacent Koonamoora Flora Reserve		9/85
19	20 km N of Port Pirie		9/85
20	2 km W of Whyalla		9/85
21	Bookaloo	3	9/85
22	30 km S of Parachilna		9/85
23	10 km E of Copley		9/85
24	Wirrealpa		9/85
25	Nullabor Plain 228 km E of Eucla		10/86
26	2 km S of Eucla	4	10/86
27	3 km E of Madura		10/86
28	10 km E of Balladonia		10/86
29	8 km N of Widgiemooltha		10/86
30	35 km S of Menzies	5	10/86
31	20 km S of Cue		10/86
32	Lake Annean		10/86
33	150 km S of Carnarvon		10/86
34	30 km S of Carnarvon	6	10/86
35	20 km N of Carnarvon		10/86

Although this study was conducted in saltbush stands during the post-dieback regenerative phase in 1985-1987, the absence of extensive stands of vigorous 'healthy' saltbush on the Riverine Plain made comparison of the endophytic fungal microflora of 'healthy' and 'diseased' bushes uncertain. To provide a reference point for fungi associated with healthy saltbush communities not known to have ever exhibited decline

symptoms, 154 vigorous plants were sampled from 11 sites in Western Australia and western South Australia in October 1986 (Table 1, Fig. 1).

In September 1985, dead pigface (*Disphyma clavellatum* (Haw.) R. J. Chinnock), at 'Tin Tin' Station, was observed within an area of 10-25 cm surrounding the stems of declining saltbush. Tissue from the apparent margin between the healthy and necrotic areas of the prostrate stems of 5 pigface plants was sampled.

In addition to the bladder saltbush sampled from Kitcho Station (site 11) in May, 1986, single plants of annual saltbush (*A. muelleri Benth.), glasswort (Pachycornia* sp.), and pale poverty bush (*Bassia divaricata* (R.Br.) F. Muell.) were also sampled to compare their internal mycoflora.

In June 1986, an isolated patch of bladder saltbush with 'dieback' symptoms was observed at Tupra Station (site 12, Fig. 1). Plants at the centre of the patch had bleached leaves and showed no signs of regrowth that was evident in surrounding bush as a result of recent rain. There was a gradient of regrowth in plants radiating outwards from the centre of this patch. Two plants were sampled from this 'dead' area and, on a radial transect, at 10, 20, 35 and 60 m from the centre. At 10 m, regrowth was occurring from axillary buds and general plant vigour increased at each sampling location until at 60 m bushes were healthy and there was no apparent sign of the plants having been stressed.

Isolations

Bushes were dissected on the day of collection or within 30 hours of sampling. Pieces of tissue, 5 to 10 mm long, were removed from the roots and/or from stems up to 12 cm above ground level. Older bushes with considerable secondary growth were cut transversely with a carpenters jigsaw. External tissue was removed to a depth of at least 1 mm with a scalpel blade to exclude epiphytic colonizers and, depending on the thickness of the section, surface sterilised in hypochlorite solution (1% available chlorine) for 40 to 180 secs. Sections were then rinsed in sterile distilled water, split longitudinally and plated on potato carrot agar. Plates were incubated in the dark at 25°C until fungal growth was observed. Colonies were examined microscopically and subcultures were made of representives of all fungal species present. Subcultures of slow growing fungi were made up to 38 days after isolations commenced.

Cultures were grown on potato dextrose (PDA) and carnation leaf (CLA) agars (Burgess *et al.*, 1988a) in the presence of near-UV light to induce sporulation. All *Fusarium* cultures were grown from single spores according to the method of Burgess *et al.* (1988a). *Phoma* spp. were grown on oat and malt agars under the conditions described by Sutton (1980). Isolation data were compared using a log linear model assuming a Poisson distribution. Marginal effects of region and fungus were removed and the zone by fungi interaction tested.

Pathogenicity testing

(i) Production of saltbush seedlings. Inflorescences collected from bushes growing near Hay, NSW were dried and the bracts removed by hand. Seeds were placed on moist cotton wool at 25°C and at the first sign of germination were planted into pasteurised soil. Soil was from the surface 'A' horizon of duplex soils near Hay, sieved and steam/air treated at 60°C for 30 minutes. Seedlings were grown at 25/20°C day/night in temperature-controlled glasshouses.

(ii) Inoculum. The experiments were conducted in October 1985. The species chosen (*Phomopsis* sp., *Libertella* sp. and *Fusarium lateritium*, Table 7) were those most frequently isolated from field specimens at the time. Vermiculite cultures of the fungi were prepared as follows. Vermiculite (20 g) was moistened with malt extract (60 ml of 3% solution of Oxoid Malt Extract), autoclaved in Erlenmeyer flasks and inoculated

with a 1 cm diameter agar plug cut from the periphery of a 7 day old culture growing on the appropriate medium. Flasks were incubated at 25°C for 14 days and shaken daily to distribute hyphal clumps.

(iii) Inoculation. A layer of vermiculite culture ca 1 cm deep was placed 2 cm below the soil surface in 9 cm plastic pots. A one-month old saltbush seedling was transplanted into each pot after adhering soil had been washed from its roots. Twenty seedlings were planted intact and another 20 after the distal half of their root system was removed with sterile scissors. Control treatments consisted of an equal number of seedlings treated as above but without vermiculite inoculum. The plants were grown in a temperature-controlled glasshouse at 25/20°C for 5 months and were watered on an alternating 3 and 4 day cycle with an equal volume of water. The alternating 4 day cycle was designed to impose moderate and repeated moisture stress to the plants. A second experiment was conducted using *Fusarium equiseti* (2 isolates), *F. nygamai* and *F. oxysporum* in January 1986 and grown for 7 months. Treatments were separated by perspex screens to prevent cross-contamination. Plants were not watered during the final 10 days of either experiment to impose an additional stress.

(iv) Examination. Five plants from every treatment were selected at random and soil was washed from the roots. The plant was sectioned below ground level into two 7.5 mm lengths; segment one, 7.5-15 mm below ground level, and segment 2, ground level to 7.5 mm deep. The stem was sectioned into three 10 mm lengths:- segment 3, 10-20 mm; segment 4, 30-40 mm; and segment 5, 50-60 mm above ground level respectively. Each segment was surface-sterilised, 2 mm was discarded from each end, and the remaining 6 mm segment was dissected longitudinally. Each piece was plated on PDA as described above for field isolations. The above ground portion of the remaining 30 plants was weighed and dried to constant weight at 60°C.

Results

Isolations

Seventy one fungal species in 40 identifiable genera were isolated from internal root and stem tissue of bladder salt bush sampled from the six geographic regions across southern Australia which correspond approximately to the chenopod communities defined by Graetz and Howes (1979). All fungi isolated are listed in Table 2 and herbarium accession numbers for representative isolates are listed in Table 3.

The saltbush communities sampled can be divided into eastern 'dieback' regions, 1 to 3, and western 'healthy' regions, 4 to 6 (Table 1). Eight of the 25 genera (*Chaetophoma*, *Coniosporium*, *Gilmaniella*, *Harknessia*, *Illosporium*, *Melanospora*, *Phomopsis* and *Sordaria*) isolated from the 116 roots and 30 stems sampled from regions 1 to 3 were not found in regions 4 to 6, whereas 15 of the 32 genera isolated from the three western regions were not detected in bushes from the eastern communities. However, except for *Phomopsis*, these seven genera were infrequently isolated in regions 1 to 3 and in most cases the listing in Table 2 represents an isolation from only one or two stems or roots. Moreover they are not recognised pathogens of woody perennials.

Fusarium species were the most frequently isolated fungi. With the exception of *Fusarium lateritium*, all the most frequently isolated fusaria were root inhabiting. *F. nygamai*, *F. oxysporum* and *F. equiseti* were the most frequently isolated species from roots and *F. nygamai* and *F. oxysporum* were generally more common in plants from regions 1 to 3. The frequency of the microconidial *Fusarium* species are compared in Fig 2. The unidentified fusaria from W.A. (Table 2) may represent three undescribed species.

Fusarium lateritium sensu Snyder and Hansen (Burgess *et al.* 1988a) was the only *Fusarium* species consistently isolated from stems and was recorded from 50% of all stems

TABLE 2

Fungal species and frequency of their isolation, from roots and stems of bladder saltbush sampled from 6 geographical locations

* Libertella spp. A, B, C and Phomopsis spp. 1, 2, 3 are possibly undescribed species; their separation is based on differences in morphology of the conidium. Superscript letters in region one denote fungal species also isolated from [a] annual saltbush, [b] glasswort, [c] poverty bush and [d] pigface.

	Region											
Number of sites sampled	1		2		3		4		5		6	
	12		5		7		4		4		3	
Number of samples	Roots 58	Stems 30	Roots 26	Stems 0	Roots 32	Stems 0	Roots 56	Stems 56	Roots 56	Stems 56	Roots 42	Stems 42
Deuteromycotina												
Hyphomycetes												
Acremonium sp.	1											
Alternaria alternata (Fr.) Keissler		4[d]			2		2	2	2	1		
Alternaria chlamydospora Mouchacca		1[a]						2	1	2		7
Aphanocladium album (Preuss) W. Gams									1	3	2	1
Aspergillus fumigatus Fres.	1	2			1		8	3	15		7	1
Aspergillus terreus Thom											1	
Aspergillus sp.	1	1					1					
Aureobasidium pullulans (de Bary) Arnaud	[b]						1			1		1
Cladosporium sp.							2					
Coniosporium sp.	1											
Curvularia sp.							1				1	
Cylindrocarpon sp.								1				
Dendryphion sp.							1					
Drechslera desmatioidea (Bubak & Wroblewski) Subram. & Jain									2			
Drechslera sp.									1		2	
Fusarium acuminatum Ell. & Ev.	1							2				
Fusarium anguioides Sherb.									1			
Fusarium avenaceum (Fr.) Sacc.	1						1					
Fusarium chlamydosporum Wollenw. & Reinking			1		1							
Fusarium compactum (Wollenw.) Gordon							1					
Fusarium equiseti (Cda.) Sacc.	17[abc]	10[d]	1		4		16	3	5	2	16	2
Fusarium flocciferum Corda							1					
Fusarium graminearum Schwabe		1										
Fusarium lateritium Nees emend. Snyder & Hansen	8	22[d]	3		5		2	15	2	21	5	34
Fusarium subglutinans (Wollenw. & Reinking) Nelson, Toussoun & Marasas							5		8	1		

TABLE 2 (continued)

	Region											
	1		2		3		4		5		6	
Number of sites sampled	12		5		7		4		4		3	
	Roots	Stems	Roots	Stems	Roots	Stems	Roots	Stems	Roots	Stems	Roots	Stems
Number of samples	58	30	26	0	32	0	56	56	56	56	42	42
Fusarium nygamai Burgess & Trimboli	26		12		13		4		15		15	1
Fusarium oxysporum Schlecht. emend. Snyder & Hansen	17[bcd]		3		4		9	1	4		2	
Fusarium redolens Woolenw.	2[c]				1		13		1		1	
Fusarium scirpi Lambotte & Fautr.	8	2			1			1		1		
Fusarium solani (Mart.) Appel & Woolenw. emend. Snyder & Hansen							1					
Fusarium sporotrichioides Sherb.									1			
Fusarium spp. undetermined							7	7	2	7	1	
Geniculosporium sp.									1			
Gilmaniella humicola Barron		1										
Illosporium sp.	1											
Paecilomyces sp.							1		1	1		
Papulospora sp.	5							1				
Penicillium spp.							2	1			1	1
Phialomyces sp.								1				
Phialophora sp.												1
Rhizoctonia sp.										1		1
Scytalidium thermophilum (Cooney & Emerson) Austwick									8	1		
Scytalidium sp.	1		1		1							
Trichoderma sp.	5	5						1				
Ulocladium sp.					1							1
Coelomycetes												
Ascochyta caulina (P. Karst.) v.d. Aa and Kest.	1	9[a]					1	8		23	1	17
Camarosporium sp.		13[abcd]			2			3		15		5
Chaetophoma sp.	1	1	1									
Coniothyrium sp.	1	15[a]			2			7		12		12
Cytospora sp.		1					2	1	2	5		1
Diplodia sp.										2		
Harknessia sp.	1											
Libertella species A*	8	5	3		8	1	8	1	1		4	
Libertella species B*	1[c]						1	4	3			

TABLE 2 (concluded)

	Region											
Number of sites sampled	1		2		3		4		5		6	
	12		5		7		4		4		3	
Number of samples	Roots	Stems	Roots	Stems	Roots	Stems	Roots	Stems	Roots	Stems	Roots	Stems
	58	30	26	0	32	0	56	56	56	56	42	42
Libertella species C*	1											
Phoma prunicola (Opiz) Wr. & Hochapf.											2	1
Phoma variospora v.d. Aa & v.d. Kest	4	1			3		1	1	1	18	3	8
Phoma spp.	1	a					5	7	7	6	3	2
Phomopsis species 1*	2						2	3		1	1	2
Phomopsis species 2*	1		1		3							
Phomopsis species 3*	2	1			1							
Pyrenochaeta terrestris (Hansen) Gorenz, Walker & Larson	5				1		1					
Sphaeropsis sp.												
Undetermined genera	c									1		1
Ascomycotina							5	9	1	1	3	1
Melanospora sp.	1	1										
Pleospora herbarum (Fr.) Rabenh.												2
Pleospora obtusa (Fuckel) V. Hochn											1	
Pleospora phaecomoides (Berk. & Br.) Winter								1				
Pleospora spp.	ad	3a					1	2		2		
Pleosporaceae undetermined genera							1		4			
Sordaria sp.		2										
Sporormiella intermedia (Aversw.) Ahmed & Cain		1	1				4	16		1		
Undetermined genera		1					2	1				

sampled. All the eastern isolates from regions 1 to 3 fit the description of *F. lateritium* Nees ex Link var. *longum* Wollenw. whereas 62% of the isolates from western regions 4 to 6 closely resemble *F. stilboides* Wollenw. var *stilboides* (Gerlach and Nirenberg 1982), differing from the former in the production of a dark purple pigment. *F. lateritium* reportedly causes dieback and twig canker in a range of woody hosts (Domsch *et al.* 1980) and *F. stilboides* causes bark and fruit diseases chiefly on citrus and coffee (Gerlach and Nirenberg 1982).

TABLE 3

Predicted isolation frequency of the most common fungi from saltbush roots in the eastern (dieback) and western (healthy) regions

Fungus	Regions 1-3	Regions 4-6
F. compactum	10.2 ± 2.2	11.5 ± 2
F. lateritium	7.4 ± 1.9	2.8 ± 0.9
F. nygamai	23.5 ± 3.4	10.5 ± 1.9
F. oxysporum	11.1 ± 2.3	4.6 ± 1.2
F. redolens	1.4 ± 0.8	4.6 ± 1.2
F. subglutinans	0	4.0 ± 1.1
Other fusaria	4.2 ± 1.4	4.0 ± 1.1
Libertella sp. A	8.8 ± 2.0	4.0 ± 1.1
Phoma variospora	3.2 ± 1.2	4.6 ± 1.2
Aschochyta	4.6 ± 1.5	6.5 ± 1.5
Camarosporium, Coniothyrium, Libertella B, C, Phoma prunicola, Sporormiella		

Although *F. lateritium* in the wider sense has been recorded in temperate as well as tropical regions, *F. lateritium* var. *longum* and *F. stilboides* are exclusively tropical and subtropical in distribution (Gerlach and Nirenburg 1982). In this study, the highest incidence of the species was in the subtropics at region 6. Ninety five percent of plants from region 6 yielded *F. lateritium* and 85% of these were of the type classed as *F. stilboides* (Fig. 3). Occasionally isolates lost the ability to produce the dark pigment rendering them indistinguishable from *F. lateritium* var. *longum*. For this reason the taxon *F. lateritium sensu* Snyder and Hansen is preferred. The perfect state of this species was not observed and attempts at 'mating' 15 representative isolates with each of four spermatizing cultures following the methods of Lawrence *et al.* (1985) were unsuccessful.

Although stems were not sampled in regions 2 and 3, there is no obvious trend in the incidence of *F. lateritium* between the eastern dieback, and healthy western, communities.

There was no significant difference between the presence of fungi in stems in either the eastern or western regions. There was, however, a significant difference ($\chi^2 = 45.27$, 9 d.f. $p < 0.001$) between the isolation frequency of fungi present in roots from the eastern 'dieback' zone and the western 'healthy' zone. Analysis of data in Table 2 predicted significantly greater occurrence of *F. lateritium*, *F. nygamai*, *F. oxysporum* and *Libertella* spp. A. in the eastern zone (Table 3) and greater frequency of *F. redolens* and *F. subglutinans* in the western zone.

There was no particular association between fungi and stems. *Phoma prunicola* was more frequent in the healthy western communities and was not isolated from plants in regions 1 to 3 (Table 2). *P. variospora* was isolated from all areas except region 2. This may be due to a more favourable micro environment within stems or merely a reflection of differences in species distribution. *Camarosporium* was more prevalent in bushes from the Hay Plain where most stems sampled were less vigorous or dead (associated with 43% of

stems), than in region 5 (27%) where plants showed no obvious symptoms. The *Camarosporium* isolates appear to represent a single species which does not fit any published descriptions of *Camarosporium* species found on *Atriplex* (M. Priest, Herb.DAR, pers. comm.)

TABLE 4

Herbarium accession numbers of representative isolates of fungi isolated from internal root and stem tissue of bladder saltbush.
* Isolates are deposited in the Australian Collection of Plant Pathogenic Fungi and Bacteria (Herb. DAR) at BCRI, Rydalmere 2116, NSW; ** see text; *** Lyophylised cultures deposited in Plant Pathology Culture Collection, AR&VC, Orange 2800, NSW

Fungi deposited	Region 1	2	3	4	5	6
Deuteromycotina						
Hyphomycetes						
Alternaria alternata	DAR*68334				68349	
Alternaria chlamydospora	68335				68346	
Fusarium chlamydosporum					68343	
Fusarium equiseti	56175					68340
Fusarium lateritium				68338	68348	68339,68342
Fusarium nygamai	55812	55814	55741b		68344	
Fusarium oxysporum	55813		56174	68355	68351	
Fusarium redolens					68359,68366	
Fusarium scirpi				68363	68350	
Fusarium spp. undetermined				68360-68362	68352,68353	68341
Coelomycetes						
Ascochyta caulina	56228c			68357		
Camarosporium sp.	56227a,56223		68331	68354		
Coniothyrium sp.	56227b			68358		
Harknessia sp.	56233a					
Libertella species A**	55738,55739		55742 to	68368		
	55749,55750		55748			
Libertella species B**	68332,68337					
Phoma prunicola				68367	68345	
Phoma variospora	56236b,68333			68364		
Phomopsis species 1**	56228a					
Phomopsis species 2**	145***					
	250***					
Phomopsis species 3**	61***					
Undetermined genus				68356		
Ascomycotina						
Pleospora sp.	68336					
Sordaria sp.	56236a					
Sporormiella intermedia				68365		

The unidentified *Phomopsis* species (Table 2) were divided into three groups based on conidial morphology:— group 1, conidia creamy yellow in mass, α conidia 7-8 x 2.5 μm mostly pointed at one end, β conidia absent; group 2, conidia white to cream in mass, α conidia 5-7 x 2.5-3 μm, more ellipsoid than group 1,β conidia absent; group 3, conidia white to pale cream in mass, α conidia up to 15 x 2-3 μm, occasionally truncate, β conidia 19-24 x 1-1.5 μm. The *Libertella* spp. were similarly grouped:— group A, conidia 20-40 μm x 1 μm; group B, 15-17 μm x 1-1.5 μm; group C, 10-12 μm x 2 μm.

A wider range of fungi was isolated at 'Tin Tin' Station from healthy bushes in autumn than in spring (Table 5), which may reflect the more stressed nature of the

bushes after a hot dry summer. Regenerating bushes were colonized by the largest number of species. *Fusarium* species were again the most frequently isolated fungi. Except for single isolations of *F. avenaceum* and *F. graminearum*, the *Fusarium* species were common to all plant categories. Species of *Acremonium*, *Chaetophoma*, *Coniosporium*, *Cytospora*, *Harknessia*, *Melanospora*, *Phomopsis*, *Pleospora*, *Sordaria*, *Trichoderma* and *F. avenaceum* and *F. graminearum*, were not isolated from 'healthy' bushes but the relative frequency of isolation of these fungi was low and in most cases their occurrence in 'dead' and regenerating bushes was limited to single isolations.

TABLE 5

The association of plant vigour with the relative frequency of isolation of fungi from internal tissue of bladder saltbush sampled on two occasions from Tin, Tin, NSW

* number of samples from which the fungus was isolated.

Date sampled	3/9/85				18/3/86					
Plant vigour	Healthy		Regenerating		Healthy		Regenerating		Dead	
No. of samples	2	0	9	4	4	3	3	3	4	3
	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem
Deuteromycotina										
Hyphomycetes										
Acremonium sp.			1*							
Alternaria alternata						1		1		
Aspergillus fumigatus							1			
Aspergillus sp.						1				
Coniosporium sp.							1			
Fusarium avenaceum			1							
F. equiseti			5	3	3		1	2	2	1
F. graminearum				1						
F. lateritium			1	1	1		1	3	2	4
F. nygamai	1				1		1		3	
F. oxysporum	1		5		1		1		3	
F. scirpi					1		3	1	2	1
Gilmaniella humicola						1				
Trichoderma sp.				2					2	
Coelomycetes										
Ascochyta caulina						1		2		1
Camarosporium sp.						3		2		
Chaetophoma sp.							1	1		
Coniothyrium sp.						2	1	2		2
Cytospora sp.								2		
Harknessia sp.							1			
Libertella sp. A							1	2	1	1
Libertella sp. B					1					
Phoma variospora					1	1	2	1	1	1
Phoma sp.						1				
Phomopsis sp. 1							1			
Phomopsis sp. 3			2	1						
Pyrenochaeta terrestris	1		1		1					
Ascomycotina										
Melanospora sp.									1	
Pleospora sp.								1		1
Sordaria sp.										1
Undetermined genus										1

All species obtained from pigface, annual saltbush, glasswort and poverty bush plants, with the exception of *Drechslera australiensis* (Bugnicourt) Subram. & Jain ex M. B.

Ellis, were also components of the fungal flora from saltbush (Table 2). *Fusarium oxysporum* was recovered from root tissue. The undetermined genus of coelomycetes isolated from plants in region 4 appears to be the same as that isolated from poverty bush in region 1.

TABLE 6

Comparison of internal mycoflora of bladder saltbush on a 60 metre transect across 'dead' and 'healthy' bushes at Tupra Station, NSW

* the number of sampled bushes (2 per sample point) from which the fungus was isolated.

	Root					Stem				
Sample point (m)	0	10	20	35	60	0	10	20	35	60
Deuteromycotina										
Hyphomycetes										
Alternaria alternata										1
Fusarium equiseti	2*	2				1	2			
Fusarium lateritium			1			2	2	2	2	2
Fusarium nygamai		1	2	2	2					
Fusarium oxysporum	1		2	2	2					
Fusarium scirpi	1	1								
Illosporium sp.				1						
Papulospora sp.	2	1			2					
Trichoderma sp.		1								
Coelomycetes										
Ascochyta caulina									1	2
Camarosporium sp.							1		2	1
Coniothyrium sp.								1	1	1
Libertella sp.								1	1	
Phoma sp.									1	
Pyrenochaeta terrestris		2								
Ascomycotina										
Sordaria sp.						1				

The fungi associated with 'dead' and regenerating plants at Tupra Station are shown in Table 6. *Fusarium equiseti*, *F. scirpi*, and species of *Papulospora*, *Pyrenochaeta* and *Sordaria* were the only fungi limited to less vigorous bushes. *F. equiseti* was isolated from both stem and root tissue of plants showing the least regeneration. *F. lateritium* was found in the stems of all plants. *F. nygamai* was associated with all plants except those from the start of the transect and *F. oxysporum* was isolated from both vigorous and unregenerating plants. A known pathogen (*Ascochyta caulina*), and two suspected pathogens of *Atriplex*, *Camarosporium* and *Coniothyrium* spp. were associated with stem tissue of the more vigorous 'healthy' plants (Table 6). With the exception of one plant with *Camarosporium* species, these fungi were not isolated from plants at the two sample points closest to the centre of the 'dieback' patch. Their presence on agar plates, however, may have been masked by the faster growing *F. equiseti*. Alternatively this may be an example of mutual exclusion of some fungal species within host tissue (Fisher and Petrini, 1992) or a succession of endophytes (Carroll *et al.*, 1977).

Pathogenicity tests

Premature chlorosis of the basal leaves appeared within 7 to 10 days of transplanting, in many plants in all treatments, but particularly in those inoculated with *Libertella* or *Phomopsis* spp. These leaves were shed over the following 3 weeks. Plants inoculated

TABLE 7

Results of pathogenicity tests with selected fungi on bladder saltbush seedlings

* Segments 1 and 2, 7.5-15 mm and 0-7.5 mm below ground level; segments 3, 4, 5, 10-20 mm, 30-40 mm and 50-60 mm above ground level respectively.

Inoculum	No. of plants at end of experiment		Plant number and condition	No. of plants from which fungus was reisolated				
	alive	dead	when sampled	plant segment *				
				1	2	3	4	5
				Experiment A — 5 months growth				
Phomopsis (type 3)	2	38	alive 2 dead 8	Not re-isolated				
Libertella (type a)	19	21	alive 6 dead 4	Not re-isolated				
F. lateritium	*9*	*31*	dead 2	2	1	0	0	0
			alive 8	0	2	4	1	0
Control	17	23	alive 5	Mostly *Gilmaniella* sp. isolated				
			dead 5	Mostly *F. oxysporum* and *F. nygamai* isolated				
				Experiment B — 7 months growth				
F. equiseti (isolate 59)	36	4	alive 10 dead —	8 —	9 —	10 —	0 —	10 —
F. equiseti (isolate 79)	36	4	alive 9 dead 1	6 1	7 1	1 1	0 0	0 0
F. nygamai	31	9	alive 7	7	6	1	1	0
			dead 3	3	3	3	2	1
F. oxysporum	36	4	alive 8	6	7	1	0	0
			dead 2	2	2	2	1	0
Control	23	17	alive 5	Mostly *Gilmaniella* sp., occasionally *F. equiseti*				
			dead 5	Mostly *F. nygamai*				

with *F. equiseti* (isolate 59), *F. oxysporum* or *Libertella* sp. grew noticeably larger and were significantly heavier and taller than the controls (Table 8). Except for the post-transplanting shock from which all treatments recovered, no obvious stress symptoms developed during the experiments until moisture stress was applied 10 days before sampling. This stress most affected the untreated controls; leaves on most plants became bleached and appeared to die. (Table 7).

TABLE 8

Mean fresh and dry weights of bladder saltbush plants, inoculated for pathogenicity testing, after 5 and 7 months

Inoculum	Mean fresh weight (g)	s.e.	Mean dry weight (g)	s.e.
	Experiment A — 5 months growth			
Phomopsis sp. (type 3)	0.926	0.232	0.676	0.239
Libertella sp. (type A)	2.002	0.740	1.220	0.209
Fusarium lateritium	1.648	0.656	1.197	0.288
Control	1.481	0.522	1.010	0.279
	Experiment B — 7 months growth			
F. equiseti (isolate 59)	3.220	0.842	1.717	0.355
F. equiseti (isolate 79)	2.871	0.559	1.441	0.272
F. nygamai	2.683	0.687	1.468	0.205
F. oxysporum	3.444	0.515	1.847	0.211
Control	2.335	0.878	1.419	0.289

As there was no apparent effect from root pruning, the reisolation frequency of fungi is combined for both treatments in Table 7. *Phomopsis* and *Libertella* spp. were not reisolated from any plant. The Fusarium spp. were always reisolated from tissue at/or below ground level and had invaded the stems or progressed acropetally more frequently in plants which were dead when sampled. There was no significant difference between plant condition (dead or alive when sampled) and the segment from which fungi were reisolated, but there was a significant segment by inoculum interaction ($\chi^2 = 31.39$, $p < 0.01$).

DISCUSSION

Isolations

A diverse range of fungi, including many known plant pathogens, was found associated with internal shoot and root tissue of bladder saltbush and other species. With the exception of 5 species (*Ascochyta caulina*, *Aureobasidium pullulans*, *Camarosporium* sp., *Coniothyrium* sp. and *Pleospora* sp.) no fungus listed in Table 2 has previously been recorded on *A. vesicaria*. In addition this is the first record of *Alternaria chlamydospora*, *Libertella* spp., *Phoma variospora*, and *Sporormiella intermedia* in Australia.

The greater number of genera isolated from regions 4 to 6 (Table 2) may simply reflect the larger number of stems sampled from these regions than the presence of a more diverse endophytic microflora.

Fusarium nygamai, a recently described species (Burgess and Trimboli, 1986) was isolated from >40% of roots from each of the known dieback regions and was relatively common in roots from region 6. It has been isolated previously from soil debris in arid regions of Queensland (Burgess *et al.*, 1989) and from both undisturbed and cultivated soils in South Africa (Marasas *et al.*, 1988). It is more commonly found in undisturbed soils under natural vegetation and forms only a minor component of the *Fusarium* mycoflora in wheat soils or on wheat plants with crown rot (van Wyk *et al.*, 1987).

Although *F. nygamai* has been frequently isolated from diseased plant tissue, its pathogenicity to plants has not yet been demonstrated.

F. oxysporum and *F. solani* are among the most widespread and predominant *Fusarium* species in uncultivated soils (Stoner, 1981). However, we recorded only a single isolate of *F. solani* from saltbush roots and *F. oxysporum* was associated with only 20% of roots sampled from the dieback regions. In contrast, *F. equiseti* which is known to occur in desert soils in the U.S.A. (Stoner, 1981) and Israel (Joffe and Palti, 1977), and is abundant in semi-arid rangelands in eastern Australia (Burgess, 1981; Burgess and Sommerell, 1992), was isolated more frequently than *F. oxysporum* in regions 4 to 6. Although it is a vigorous saprophyte, *F. equiseti* has occasionally been implicated in disease (Burgess *et al.*, 1988b).

In a recent survey of *Fusarium* species in soils along a longitudinal transect from Darwin to Ceduna, Sangalang *et al.* (1991) isolated *F. equiseti* and *F. oxysporum* most commonly from semi-arid soils in the Ceduna area (region 4). During the present study *F. chlamydosporum*, *F. compactum*, *F. nygamai*, *F. scirpi* and *F. subglutinans* were isolated from roots in regions 4 and 5. It is perhaps noteworthy that these species were not recorded in soils by Sangalang *et al.* (1991) from the climatically similar region of Ceduna, and that they isolated *F. subglutinans* only from Darwin (tropical) soils. In a recent study, however, Burgess and Sommerell (1992) isolated *F. chlamydosporum* most frequently from drier soils in western Queensland.

Fusarium nygamai has features in common with *F. oxysporum* and *F. subglutinans* to which it is related. It is therefore likely that it behaves in a similar way to other fungi in this group which move up through the host plant by microspores carried in the xylem. It is thus possible that these four species from saltbush communities across Australia occupy a similar ecological niche.

When the frequency data for *F. oxysporum*, *F. redolens*, *F. subglutinans* and *F. nygamai* is combined for each region (Fig. 2), region 1 (the dieback area on the Hay plain) has noticeably more plants infected with one or more of these fungi. Although there were fewer plants infected with these four *Fusarium* species in the healthy communities in regions 4 to 6, >40% of roots sampled were colonised by one or more of these species. When considered with the pathogenicity testing, however, there is insufficient evidence to suggest a direct causal relationship between these fungi and saltbush dieback. *F. oxysporum* has, however, been isolated from wilting galvanised burr (*Bassia birchii* (F. Muell.) F. Muell.) growing in western NSW and its pathogenicity has been demonstrated (Auld, 1976). This suggests that in semi-arid environments, *F. oxysporum* could potentially influence saltbush vigour.

Several fungi have been isolated from *Atriplex* species in Australia but their pathogenicity is unproven. *Coniothyrium atriplicinum* Wint. and a number of *Camarosporium* spp. were isolated from leaves and stems of unthrifty and healthy cultivated and naturally growing *A. undulata* and *A. rhagodioides* (= *A. amnicola*) in Western Australia (Napier, 1983). She found no clear correlation between symptoms of disease and the presence of either fungus. Neither fungus induced disease symptoms in inoculated plants, although *C. atriplicinum* was reisolated from all inoculated plants. Napier observed that because of the range of growth habits, it was difficult to know what a 'normal' plant should look like. It is interesting to note that Napier (1983) isolated *Aureobasidium pullulans* from declining *Atriplex* spp. This fungus was recorded from saltbush by us only in Western Australia. In the eastern regions where bladder saltbush decline had occurred we detected *A. pullulans* only in glasswort.

Coniothyrium atriplicinum has been recorded on several *Atriplex* species in Victoria (Chambers, 1982) and from leaves and stems of *A. nummularia* in NSW (Anon., 1983), as well as in W.A. *Camarosporium* spp. have been recorded on *Atriplex* spp. in South Australia

(Cook and Dubé, 1989) and Western Australia (Shivas, 1989). *Ascochyta caulina* has been isolated from stems and crowns of *A. vesicaria* in NSW (Herb. DAR, unpub.), and from *Atriplex* spp. in W.A. as *A. chenopodii* Rostr. (Shivas, 1989). This fungus is a common cause of leaf spots and is known to cause stem necrosis on various species of *Atriplex* and *Chenopodium* (van der Aa and van Kesteren, 1979). More stems were sampled in regions 4 to 6 than in the dieback regions but the frequency of isolation of *A. caulina* approximated that from region 1, suggesting that it is unlikely to be the sole cause of saltbush dieback.

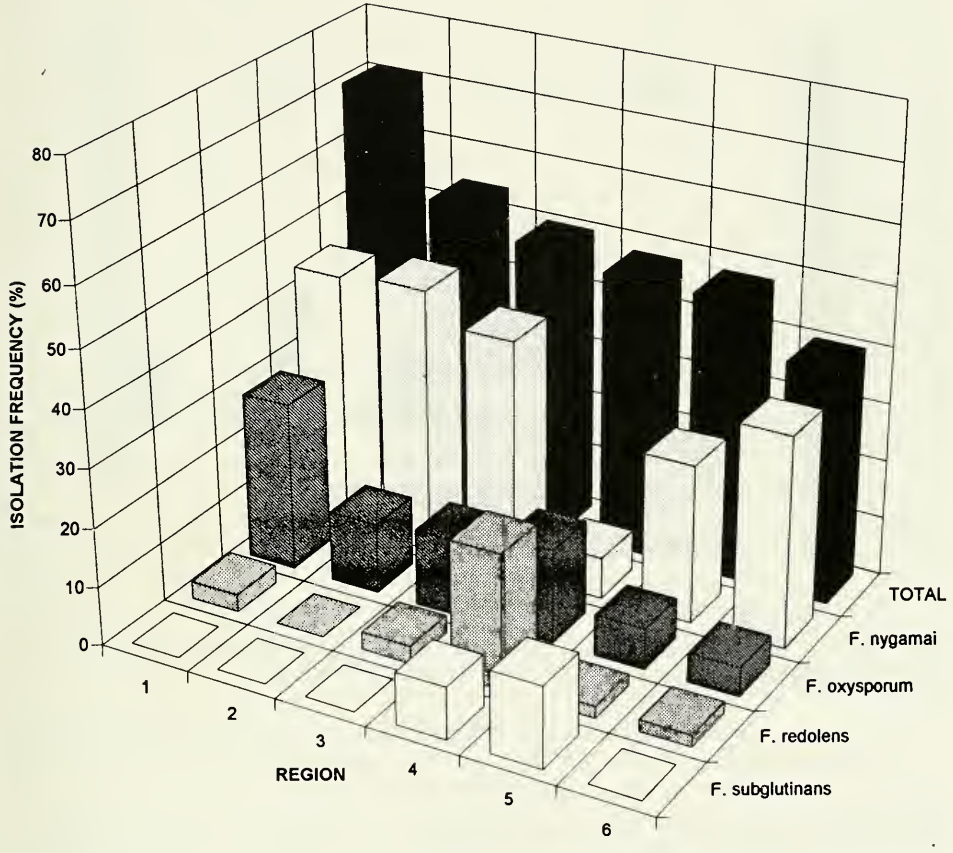

Fig. 2. The isolation frequency of microconidia-forming *Fusarium* species isolated from bladder saltbush sampled from six geographic regions, and the total proportion of sampled roots that were infected with one or more of these species.

Pathogenicity Testing

A realistic pathogenicity test involving a long-lived woody perennial is difficult to achieve in a glasshouse when several variables such as plant age, extreme temperature and rainfall patterns, fluctuating soil salinity, grazing pressure, defoliation, etc., may modify plant response to a pathogen. Under the experimental conditions imposed during the pathogenicity tests, there was no obvious relationship between a particular fungus and development of lesions or stress symptoms. The observed growth stimulation (Table 8) may be the result of a synergistic relationship between fungus and host

providing improved nutrient availablity to the plant. Cother and Gilbert (1993) recently reported growth stimulation in rice seedlings by pathogenic *Pythium* species. The endophytic microflora in plants may be beneficial in relatively unstressed situations or detrimental when host vigour is impaired.

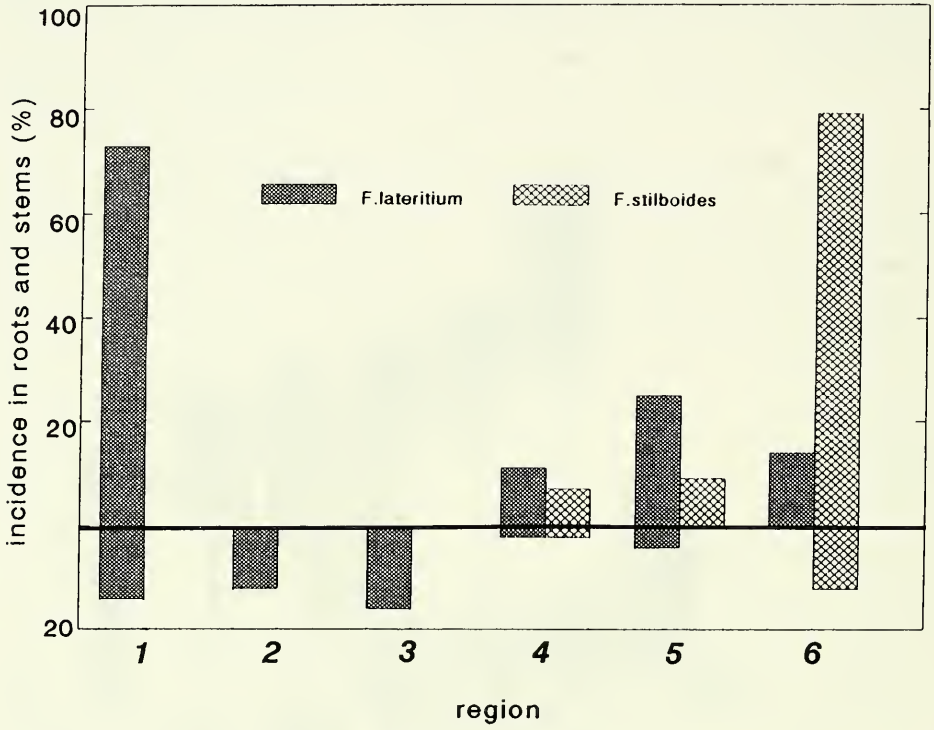

Fig. 3. The percentage incidence of *Fusarium lateritium* and *F. stilboides* in roots and stems of bladder saltbush sampled from six geographic regions. The horizontal line depicts ground level.

Fusarium oxysporum, *F. equiseti* and *Gilmaniella* spp. are ubiquitous soil inhabitants, even in desert environments (Joffe and Palti, 1977), and are somewhat tolerant of short periods of steam-air treatment. The isolation of *Fusarium* spp. from uninoculated (control) plants which had died in the experiment is attributed to the soil-borne nature of these fungi rather than to cross-contamination.

In a discussion on stem and crown cankers, and dieback of woody plants, Schoeneweiss (1981) noted that these disorders are most prevalent on plants subjected to environmental stress before symptom development. Severely defoliated plants appeared more susceptible to root rots and dieback pathogens, and several periods of repeated stress may result in a static predisposition to nonaggressive pathogens (Schoeneweiss, 1981). Manion (1981) considered that three sets of factors may be involved in tree decline with one factor from each category associated with a decline situation. A predisposing factor (e.g. climate, plant age) influenced by an incitant (e.g. drought, defoliation) together with a contributor (e.g. fungi, viruses) may culminate in the death of a tree.

Low and extremely variable precipitation is the predominant factor limiting growth in saltbush communities (Osborn *et al.*, 1980). Semple (1989) in a comparison of recorded saltbush 'dieback' events with the occurrence of drought, suggested that,

although there is some evidence of periods of unusually high rainfall preceding dieback, it could be equally argued that saltbush dieback is associated with drought. Each condition is a potential stress factor, directly affecting plant vigour or indirectly affecting physiological functions through changes in soil salinity, ion uptake, hydraulic conductivity, etc. (Cother *et al.*, 1988). For example, wilt symptoms in *Zizyphus mauritania* Lamk. were caused by *F. equiseti* only when moisture stress was imposed on inoculated plants (Lodha, 1983), and it was suggested that reduced root vigour caused by droughting may favour fungal infection.

A number of the fungi isolated from naturally grown bladder saltbush and adjacent plants during this study, e.g. species of *Acremonium*, *Aureobasidium*, *Camarosporium*, *Coniothyrium*, *Cylindrocarpon*, *Cytospora*, *Libertella* and *Phoma* are recorded in the literature as being associated with dead and dying plant tissue. Saprophytism by these species is common on senescing tissue and some records do not involve known pathogenicity (Ellis and Ellis, 1985). It is possible that the association noted in these records and their involvement in saltbush decline is one of colonization of tissue during periods of plant stress. Alternatively these and other species, and especially the fusaria, isolated from 'healthy' field specimens may reside symptomlessly in a neutral or commensal relationship in root and crown tissue, as indicated by the relative frequency of isolation of *F. oxysporum*, *F. nygamai* and *F. equiseti* from these tissues in 'healthy' inoculated plants. When the plants' normal vigour is reduced by some external (e.g. drought, grazing pressure) or internal condition (e.g. onset of senescence) such fungi may become opportunistic pathogens. Saltbush dieback may thus be the culmination of one or more events which, by altering host physiology sufficiently, predisposes the plant to its endophytic mycoflora. Similar symptomless associations of known pathogens have been reported in maize (Young and Kucharek, 1977; Windham and King, 1983) and soybeans (Müeller *et al.*, 1985; Sinclair, 1991). Recently, Fisher and Petrini (1992) isolated *F. equiseti*, *F. oxysporum* and *Phoma* spp. from healthy rice plants and similarly argued that symptomless endophytes may induce symptoms when the host is stressed. Moreover, a number of species associated with bladder saltbush were also found in rice, supporting the view of Fisher and Petrini (1992) that vascular plants have a basic predisposition to allow symptomless endophytic colonization by a number of fungi.

The tolerance of individual saltbushes to this coexistence may vary so that at any level of externally imposed stress some individuals in the population die or display 'dieback' symptoms. The stress conditions may take many forms and the involvement of climatic factors may explain the cyclical nature of this phenomenon.

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